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14. ABSTRACT: In order to implement the use of nanotools for detection and treatment inside breast channels it is critical to develop cell culture methods that mimic the mammary ductal system characterized by branched channels with decreasing lumen sizes. Here we report the first mimicry of branched mammary ducts. The branched system was first designed using soft lithography and PDMS as the molded material and then using acrylic support to permit round shapes for the duct channels. Non-neoplastic mammary epithelial cells can cover laminin 111-precoated channels and display basoapical polarity, like in the phenotypically normal situation. The acrylic based system was then successfully used to culture tumors in the presence of non-neoplastic cells lining the channels. Superparamagnetic microparticles (SMPs) were tested on phenotypically normal cells and showed lack of deleterious effects on survival and differentiation. Once PEGylated the SMPs could be readily moved along the surface of the ductal cells using a magnet. This pilot project has shown that it will be possible to test SMPs designed to target abnormal cells for detection and treatment within a breast ductal system.

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Introduction

The possibility of detecting and treating breast neoplasia via intraductal access would permit the identification of all lesions and local treatment, thus decreasing the risk of systemic side effects. However, the intraductal delivery of nanotools or probes is hampered by fluid resistance due to the decreasing size of ductal lumena. Therefore neoplastic cells located in the distal and smallest channels would not be reached. One possible way to access all preneoplastic and tumor sites in the breast might be the use of guided superparamagnetic submicron particles (SMPs). Indeed the fluid can be left static if SMPs are moved through the breast ducts under appropriate magnetic fields. In order to design appropriate SMPs, it is critical to develop a ductal system *in vitro* that would provide a template for toxicity and targeting tests. The goal of this project is to develop such *in vitro* ductal system and assess the movement of SMPs inside the ductal system (Aim 1) and design SMPs with targeting capabilities for specific cell subsets (Aim 2).

Body

Aim 1: ***To determine the potential for SMPs to travel through duct-like channels***

Task 2: *set up of monolayer mammary epithelial cultures displaying cell polarity on filters* – COMPLETED in 2010
Basoapical polarity could be obtained with non-neoplastic breast epithelial S1 cells cultured on filters; we have also confirmed that non-neoplastic breast epithelial MCF10A cells display basal polarity on filters. This represents a simplified system to study intercellular permeability of SMPs.

Tasks 7 and 8: *Design of a ductal system in vitro* –COMPLETED
One of the challenges of mimicking the phenotypically normal organization of the breast epithelium is to reproduce the backbone of differentiation, the basoapical polarity axis (Plachot et al 2009). Another challenge is to apply microscale cell culture necessary to mimic the size of the breast ducts to effectively produce mammary epithelial differentiation in serum-free medium, not by producing the well-known spherical three-dimensional (3D) structures referred to as acini, but by producing a basoapically polarized monolayer of non-neoplastic cells organized into a tube. In last year's report we demonstrated that polydimethylsiloxane (PDMS) could be used as hard mold to develop branched hemichannels of decreasing diameter which, once covered with dried laminin 111, permitted expansion of a monolayer of non-neoplastic S1 cells to 90% confluence with full basoapical differentiation. This work was published in January 2011 in Integrative Biology (Grafton et al 2011; see appendix).

A breast ductal system starts at the nipple with a main duct that reportedly has a diameter of ~100 µm, and ends with terminal ducts at the TDLU with a diameter of 5-10 µm on average. Soft lithography was used to design PDMS branched channels of decreasing sizes from 50X120 µm, to 50X60 µm and 50X30 µm (height and width) to be coated by cells of ~10 µm thickness. Each channel of a particular diameter had a length of 5 mm and the whole ductal system could fit on a slide thus, enabling on-chip experiments. Due to the tiny total surface of 7.1 mm^2 and total volume of 0.09 mm^3 of the on-chip ductal system we had found that branched hemichannels that could be closed at room temperature by adding a PDMS coverslip with a concentration of cells increased 10 fold compared to usual, was the most effective culture method and would permit the easy addition of tumor cells inside the channels.

***Progress for 2010-11*: Improved design of the breast-on-chip -** The initial breast-on-chip (Grafton et al., 2011) was engineered using PDMS molding. It enabled us to precisely control

the dimensions (width and depth) of the branched channels mimicking a breast ductal system. One limitation of PDMS molding was the production of rectangular shapes with piling up of cells in the angles. To overcome this limitation, a new production strategy has been developped based on acrylic laser micromachining. It has the advantage of reducing development cost/time; adjusting the microchannel's depth across the chip; and importantly generating semi-circular channels (**Fig. 1**). Briefly, microchannels were engraved in Clarex-cell-cast acrylic sheets (Astra-Products, NY). Out-of-focus laser was used and 950PMMA A11 (Microchem, MA) was span-coated against the microchannels. By adjusting the spinning conditions, we achieved smooth, U-shaped, microchannels. Non-neoplastic HMT-3522 S1 cells covered extensively acrylic hemichanels coated with laminin-111 (more uniformly compared with PDMS surfaces, **Fig. 1 C and D**). The acrylic hemichannels covered with cells can be sandwished between PDMS surfaces to produce a hard cast that can be easily handled for microscopy. We anticipate that in the future the acrylic platform can be further developed to achieve branching structures in a complex three-dimensional ductal tree. Note that the initial channels produced before optimisation of the technique (V-shaped or rough in texture) led to multilayered epithelia models (**Fig. 2**). This suggests that modulating the smoothness of the channels allows us to recapitulate normal-looking monolayer epithelia as well as neoplastic-like multilayers. Similar to cell culture on laminin 111-coated PDMS (Grafton et al., 2011), S1 cells in the laminin 111-coated acrylic channels were baso-apically polarized as evidenced by the basal distribution of the polarity marker α6-integrin and the apical localization of the tight-junction marker ZO-1 (**Fig. 2**).

Task 9: *Migration of SMPs inside an in vitro ductal system*-COMPLETED

One of the goals of the ductal system on-a-chip is to provide a model to study the use of fluorescent magnetic nano or submicron particles for future detection and treatment applications. In the first part of the work, SMPs of 0.86 µm in diameter were loaded into closed cell-free microfluidic channels to assess their movement in this constraint environment. It is important that these SMPs be "superparamagnetic", which means that they become magnetic only in the presence of a magnetic field. When the magnetic field is taken away the SMPs are free to disperse locally and, if targeted to surface markers on specific cells, bind their targets. After injection, the SMPs showed Brownian movement, as expected. Upon addition of the magnet, SMPs could be collected on one side of the channel or directed to enter specific channels of decreasing size (movies were made; see Grafton et al 2011 on the Journal's site).

The last step of this task was to proceed with the same experiment but this time with cell-covered channels. However, in order to do that we needed to have completed the SMPs coating (see Aim 2, tasks 1,4 & 6).***During 2010-11***, it was observed that PEGylated SMPs could indeed be moved in the presence of the cell monolayer using an externally applied magnetic field (movies were made but cannot be shown here).

Aim 2: ***To assess the capability for SMPs to travel within neoplastic lesions and identify cells with specific phenotypic traits***

Task 3: *preparation of heterogeneous tumors*-COMPLETED
In previous work we had successfully mixed ductal carcinoma in situ S2 cells with invasive T4-2 cells in 3D cultures. Cells could be recognized by GFP expression in T4-2 cells. We used a similar approach to culture growing tumors within the breast-on-chip system.

Results obtained in 2010-11: The driving force behind the engineering of the breast-on-chip is the development of early breast cancer detection and treatment methods. As shown in **Fig. 3**, triple-negative breast tumors formed by HMT-3522 T4-2 cells could be cocultured with non-

neoplastic S1 cells in the microchannels and detected using antibodies against the cancer stem cell marker CD44. This model of culture will enable us to test magnetic-guided targeting of CD44 positive breast cancer cells with SMPS.

Task 5: *Assessment of SMPs toxicity*-COMPLETED in 2010
Confocal microscopy had revealed that SMPs did not seem to enter inside cells and that there was no detectable effect of SMPs on apical polarity when used as a 10 fold concentration compared to cells (Grafton et al 2011). SMPs were used as raw materials in these experiments and therefore stuck nonspecifically to the cells and parts of PDMS noncovered by cells. This close encounter between SMPs and cells was necessary to ascertain that the SMPs per se (i.e., without any specific targeting) would not be affecting cells.

Tasks 1,4, & 6: SMPs have been chosen and tested for toxicity as explained in task 5. It took some time to identify the best particles available to be used for the proposed experiments as they had to have specific criteria like size amenable for travel inside ducts but big enough not to enter cells or pass through tight junctions, fluorescence, paramagnetic capability, possibility of conjugating antibodies. Our first attempt with antibody conjugation had not shown specificity of targeting probably due to the fact that the conjugation failed. SMPs kept binding nonspecifically to cells and laminin 111 substratum, even after incubation and wash with Bovine Serum Albumin.

Results obtained in 2010-11: We prepared PEGylated magnetic fluorescent SMPs by conjugating PEG 2000 to carboxylated SMPs. 0.1ml of SMPs suspension in ultrapure water (1 molar equivalent, 5.5 µmoles carboxyl groups based on suspension containing 55 µmoles of carboxyl groups per gram of SMPs) was incubated with three molar equivalents (16.5 µmoles, or 16.5mg) of PEG 2000. Dimethylaminopyridine (DMAP, 0.5 molar equivalents) and 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC) hydrochloride (1.2 molar equivalents) were added as zero-length crosslinker and reaction catalyst, respectively, and the reaction was allowed to stir overnight at room temperature in the dark. The reaction mixture was transferred to a dialysis membrane and dialyzed against water to remove unattached PEG. The recovered PEGylated fluorescent SMPs were transferred to a clean glass vial and stored at 4^{o}C until needed for experiments.
A Malvern Zetasizer ZS dynamic light scattering (DLS) instrument was used to measure both the hydrodynamic diameter and also the zeta potential of the stock of nonPEGylated SMPs and the PEGylated SMPs. Size measurements enabled us to assess the overall size of the hydrated PEGylated SMPs in solution and whether the PEGylation was successful as indicated by an observed size increase for the PEGylated SMPs relative to the size of the stock SMPs. Zeta potential measurements, in addition to being used to assess whether the PEGylation reaction was successful, were useful for monitoring the stability of the modified SMPs in aqueous solutions. Size and zeta potential measurements of stock SMPs and PEGylated SMPs were collected immediately after the dialysis was completed on 07/15/2011 and again on 08/02/2011. The initial measurements suggested that the PEGylation of the SMPs was successful. Although it was observed that the PEGylated SMPs could be moved in cell cultures using an externally applied magnetic field up to 30 minutes post-application of SMPs to cell cultures, moving SMPs was not possible anymore after that time. It was hypothesized that the SMPs might be “sticking” to the cells due to degradation of the PEG coating over time upon short-term exposure to various components of the cell culture medium. To test this hypothesis, PEGylated SMPs were incubated in the presence of culture supernatants (i.e., previously incubated with cells) and in

fresh medium. Approximately 100ul of these particles were incubated with 1ml of each of the media formulations in separate cuvettes. After 1hr incubation at room temperature, the particles were isolated using a magnet and resuspended in water. Samples were first measured in culture medium; however, the conductivity of the medium impaired the interpretation of the zeta potential data. Also, additional 100ul aliquots of the PEGylated particles were added during the incubation time because the initial amount added was not clearly visible when isolated using the magnet. Size and zeta potential measurements were then recorded to evaluate whether there was any apparent SMP degradation over time and reversion to non-PEGylated form (PEG degradation). Each sample was measured in triplicate. Size data were also collected (not shown) for each sample in medium prior to redispersing the samples in water. For the most part, the PEGylated SMP samples that were incubated with media exhibited zeta potential values that were more negative than the values observed for the particles that were measured in water just prior to this experiment (no exposure to medium). These values were consistent with those measured for the stock suspension of SMPs used for PEGylation. This suggests that incubation of the PEGylated particles with media (regardless of additives, with one exception; see Table 1) causes the PEG coating to be degraded with time such that it is no longer conjugated to the particles (**Table 1 and Fig. 4**).

The size data for the PEGylated SMP samples also suggest, in part, that the medium +H14, +E (i.e., medium with all additives including Epidermal Growth Factor) caused PEG "stripping" from the SMPs (**Fig. 5**). This hypothesis is based on the smaller average hydrodynamic diameters observed for these samples relative to the diameters observed for the PEGylated SMPs that were not exposed to complete media. Altogether results with incubation of PEGylated SMPs in fresh medium without additives (-H14, -E) or in fresh +H14 medium suggests that the "E" factor and also cell-related components in the media may be primarily responsible for PEG degradation. More experiments will be necessary to confirm these hypotheses.

Key accomplishments

- Developed two successful *in vitro* models of ductal breast system
- Identified paramagnetic nanoparticles that can be moved inside channels of decreasing size using a magnetic field and nontoxic to cells.
- Developed *in vitro* coculture models of tumor growing within a ductal breast system

Reportable outcomes

- Manuscript published in Integrative Biosciences: **Breast on a chip: 3D tissue engineering mimicry of the channeling system of the breast for on-chip development of nanomedical theranostics** by Meggie Grafton*, Lei Wang*, Pierre-Alexandre Vidi*, James Leary, and Sophie A. Lelièvre; (* first coauthors) with press release from Purdue University in PurdueToday.
- Abstract: **Breast ductal tree produced on-chip for the development of nanomedical tools for intraductal diagnosis and treatment of breast neoplasia** (Poster presented by Pierre-Alexandre Vidi at the Era of Hope 2011 meeting).
- Abstract: **A global endeavor towards primary prevention with the international breast cancer and nutrition (IBCN) project: Novel bioengineering-based detection and diagnostic initiatives.** Sophie A Lelièvre[1,5], Ji-Xin Cheng[2,5], Joseph Irudayaraj[3,5], James F. Leary[1,5], Connie M. Weaver[4,5], and the IBCN core committee. [1]Department of

Basic Medical Sciences; [2]Weldon School of Engineering; [3]Department of Agricultural and Biological Engineering; [4]Department of Nutrition Science; [5]Oncological Sciences Center and Center for Cancer Research, Discovery Park, Purdue University, West Lafayette, IN 47906; published in the meeting booklet of the NIH technology for Global Health meeting, Bethesda, MD, August 2011

- Manuscript in preparation on the acrylic-based model of the ductal breast system and coculture with tumor cells by the Lelièvre and Leary teams.
- PhD obtained by Meggie Grafton (Leary laboratory)
- Graduate Fellowship from the Indiana Clinical and Translational Science Institute by Juan-Manuel Cardenas-Mora in 2010 (Lelièvre laboratory).
- Dr. Lelièvre has been appointed as the Associate Director of the Purdue Center for Cancer Research for the Discovery Groups (5% commitment) in 2010 and Purdue University Faculty Scholar (2011-2016).
- Dr. Leary was selected as the recipient of the 2011 International Journal of Nanomedicine Distinguished Scientist Award. The annual award is presented to an established scientist who has made significant contributions to the field of nanomedicine.
- The grant helped support salaries for Meggie Grafton, Christy Cooper, James Leary for the Leary team and Juan-Manuel Cardenas-Mora, Lei Wang and Sophie Lelièvre for the Lelièvre team.

Conclusions

Our findings show that it is possible to use the HMT-3522 S1 breast cell model to produce a basoapically polarized monolayered epithelium on PDMS and acrylic based scaffolds as long as extracellular matrix component and differentiation inducer laminin 111 is present. PDMS and acrylic can be used to engineer branched channels of decreasing size mimicking the breast duct-like system and fit on a glass slide, and S1 cells can be cultured on hemichannels (with three of the walls) before completing the channels with the fourth wall (PDMS coverslip). To the best of our knowledge, this type of models represents the first breast ductal system on-a-chip.
Nanoparticles have been identified for fluorescence and magnetic field use. The particles can be moved in the cell free ductal system and in the presence of cells after PEGylation. Therefore these particles can be used in the future to target specific cell surface markers. Future steps include producing stable coating of paramagnetic particles. We are also thinking of developing an hemichannel system coated with parylene for instance that could be peeled off after cell culture and allow bonding of two hemichannels for easy completion of channels.

References

1. Plachot C, Chaboub LS, Adissu HA, Wang L, Urazaev A, Sturgis J, Asem EK, Lelièvre SA. Factors necessary to produce basoapical polarity in human glandular epithelium formed in conventional and high-throughput three-dimensional culture: example of the breast epithelium. BMC Biol. 2009 Nov 16;7:77.

2. Grafton* MMG, L Wang*, P-A Vidi, JF Leary, and SA. Lelièvre. “Breast on-a-chip: Mimicry of the channeling system of the breast for development of theranostics”. Integr Biol (Camb), 3: 451-9, 2011 (* first coauthors)

Supporting data

1. **Tables:**

Sample	Zeta potential mean (n=3), mV
Non-PEGylated SMP stock suspension in water	-46.2
PEGylated SMPs in water—07/15/11	+32.6
PEGylated SMPs in water—08/02/11	-13.3
PEGylated SMPs in fresh medium –H14, -E	-36.0
PEGylated SMPs in fresh medium +H14, -E	-33.7
PEGylated SMPs in fresh medium +H14, +E	-35.6
PEGylated SMPs in cell exposed medium+H14, -E	-9.52
PEGylated SMPs in cell exposed medium +H14, +E	-47.5

Table 1. Tabulated zeta potential values for non-PEGylated stock SMP solution and for PEGylated SMPs in various cell culture medium samples.

2. **Figures**

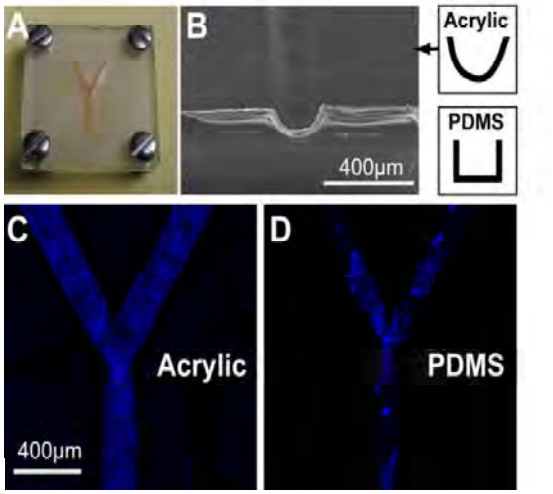

Figure 1. A Picture of branched acrylic microchannels filled with a dye. **B** Scanning electron micrograph of a laser micromachined microchannel after PMMA A4 spin coating at 4000 rpm. The result is a smooth microchannel with semi-circular cross section. This new approach is compared with the previous - PDMS based - breast-on-chip in the schematics (right). **C-D** Maximal intensity projections of confocal images showing the extensive coverage of DAPI-stained S1 cells (blue) in the acrylic compared to PDMS channels.

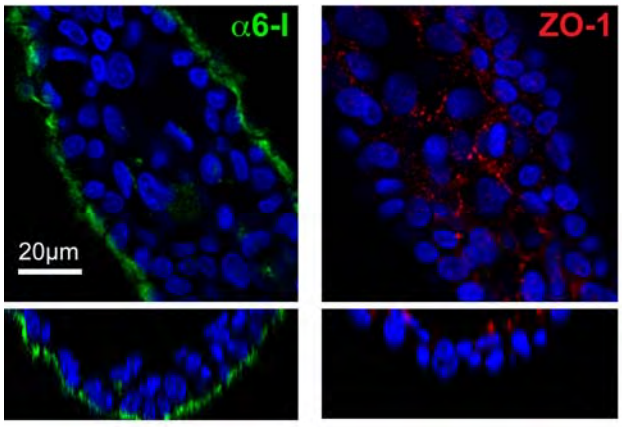

Figure 2. Polarity axis of S1 cells cultured on Laminin-111 coated acrylic hemichannels. Cells were immunostained with α6-integrin (green) and ZO-1 (red). Nuclei were counterstained with DAPI. In this particular experiment the choice of the roughness of the channel leads to double layering at certain places; whereas, other roughness conditions lead to only one layer of cells.

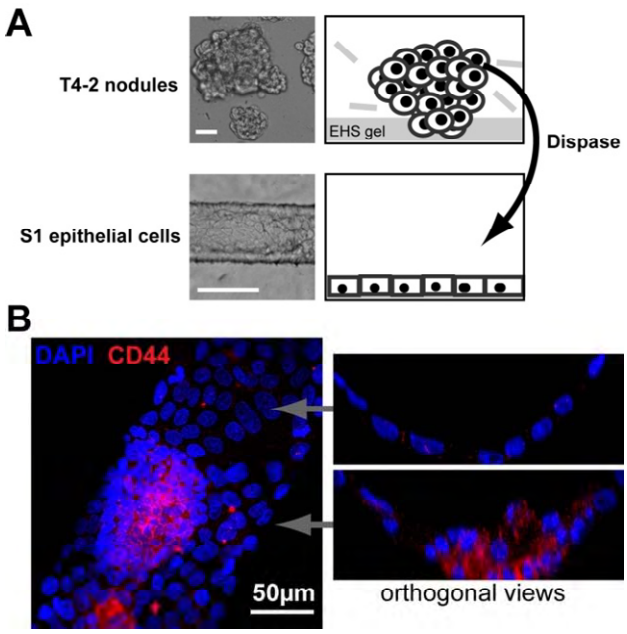

Figure 3 Coculture of non-neoplastic and malignant cells in acrylic microchannels. **A** T4-2 nodules were produced in 3D culture in the presence of Engelbreth-Holm-Swarm (EHS)-derived basement membrane components, released with dispase treatment, and added to S1 cell monolayers in acrylic microchannels. **B** CD44-expressing T4-2 cells were detected by immunostaining. The maximal intensity projection of confocal images is shown on the left. Cross-sections (orthogonal views) at different levels in the channel (arrows) are displayed on the right.

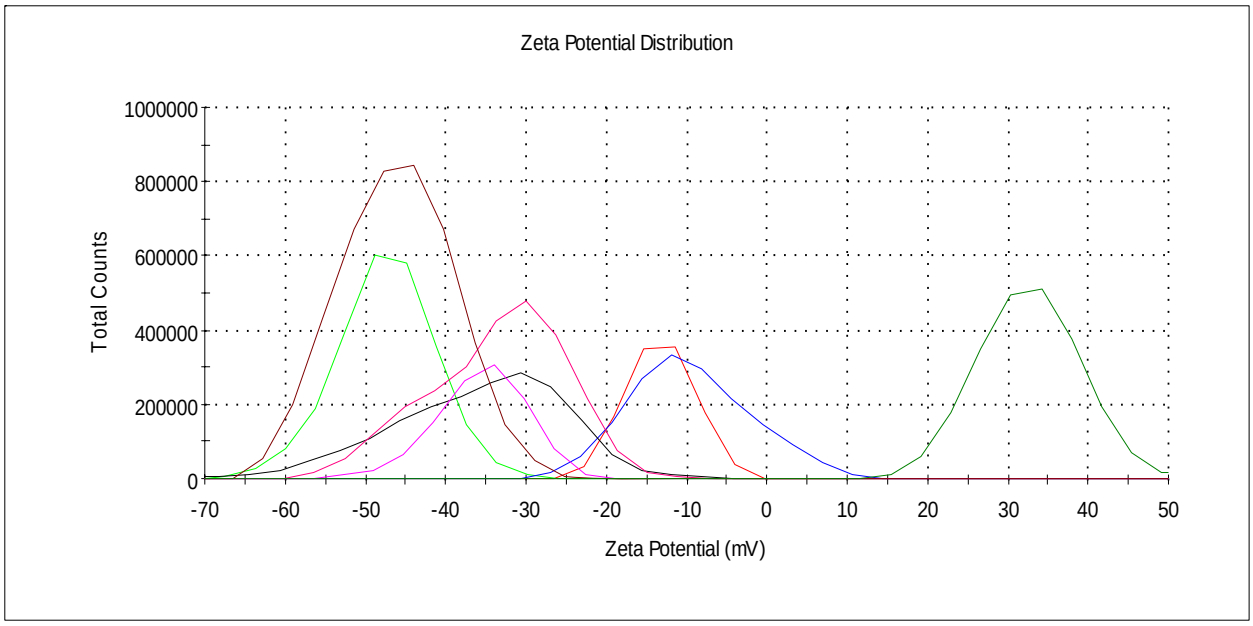

Figure 4. Zeta potential distribution data for nonPEGylated SMPs and for PEGylated SMPs incubated with various cell culture media. Brown: Stock suspension (unPEGylated), Dark green: PEGylated SMPs (measured on 07/15/11), Red: PEGylated SMPs (measured on 08/02/11), Black: PEGylated SMPs in fresh medium (-H14, -E), Pink: PEGylated SMPs in fresh medium (+H14, -E), Pink-Purple (lower of two "pinkish" plots): PEGylated SMPs in fresh medium (+H14, +E), Blue: PEGylated SMPs in medium (+H14, -E) that was exposed to cells, Light green: PEGylated SMPs in medium (+H14, +E) that was exposed to cells. H14: all additives minus epidermal growth factor; E = epidermal growth factor

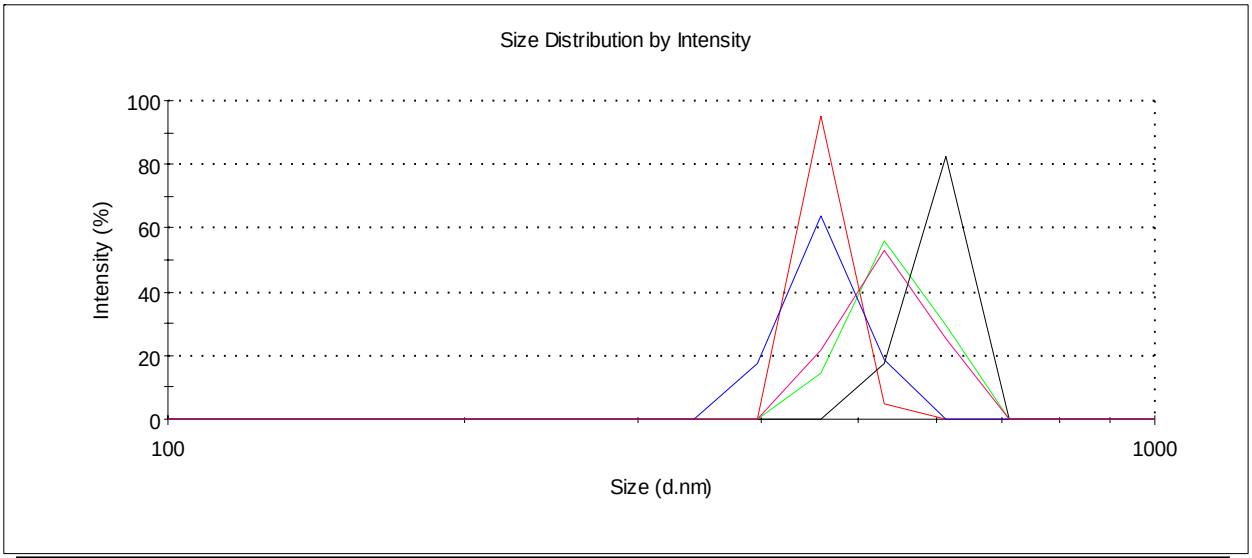

Figure 5. Intensity size distribution data for non-PEGylated SMPs and for PEGylated SMPs incubated with various cell culture media. Pink: PEGylated SMPs in water, Green: PEGylated SMPs in fresh medium +H14, -E, Black: PEGylated SMPs in fresh medium –H14, -E, Blue: PEGylated SMPs in fresh medium +H14, +E, Red: PEGylated SMPs in medium +H14, +E previously exposed to cells in culture. H14: all additives minus epidermal growth factor; E = epidermal growth factor

APPENDIX: Grafton et al 2011 publication

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PAPER

Breast on-a-chip: mimicry of the channeling system of the breast for development of theranostics†‡§

Meggie M.G. Grafton,¶[c] Lei Wang,¶[a] Pierre-Alexandre Vidi,¶[a] James Leary*[abcd] and Sophie A. Lelièvre*[ab]

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Improved detection and therapy of breast neoplasia might benefit from nanodevices traveling inside mammary ducts. However, the decreasing size of branched mammary ducts prevents access to remote areas of the ductal system using a pressure-driven fluid-based approach. Magnetic field guidance of superparamagnetic submicron particles (SMPs) in a stationary fluid might provide a possible alternative but it is critical to first reproduce the breast ductal system to assess the use of such devices for future therapeutic & diagnostic (''theranostic'') purposes. Here we describe the engineering of a portion of a breast ductal system using polydimethylsiloxane (PDMS) microfluidic channels with a total volume of 0.09 μl. A magnet was used to move superparamagnetic/fluorescent SMPs through a static fluid inside the microchannels. Non-neoplastic mammary epithelial S1 cells developed basoapical polarity as a flat monolayer on the PDMS surface when cultured in the presence of laminin 111, and incubation with SMPs did not result in detectable toxicity. Cells could not withstand the fluid pressure if microinjected directly in completed channels. Whereas, they readily covered laminin 111-coated PDMS surfaces when cultured in U-shaped ''hemichannels'' before completing the channels. This breast-on-chip model represents a critical step towards the mimicry of the tree-like ductal system of the breast for further testing and targeting of SMPs.

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† Published as part of an *Integrative Biology* themed issue in honour of Mina J. Bissell: Guest Editor Mary Helen Barcellos-Hoff.

‡ This article is dedicated to Mina J. Bissell for her visionary influence on multidisciplinary research and the necessary development of meaningful cell culture systems.

§ Electronic supplementary information (ESI) available. See DOI: 10.1039/c0ib00132e

¶ The authors contributed equally to the work.

Introduction

The human mammary gland is an assembly of several branched ductal systems with channels of decreasing size.[1–3] All luminal epithelial cells are thought to be in contact with the central lumen of the ducts, thus early alterations occurring at the apical side of cells (*i.e.*, against the lumen) and small tumors might already be detectable if there is intraductal access. An approach referred to as ductal lavage has been used to collect loose cells from mammary channels with the goal of detecting cancer cells early.[4] Although ductal lavage and even nipple aspirates can be successfully used to identify abnormal cells,[5] these techniques are likely limited to restricted

Insight box

We report the development of a new cell culture model that mimics a portion of the breast ductal system. This system will bring the possibility of studying tumor nodules inside ducts of given sizes and testing submicron- and nanoparticles for the detection and treatment of tumor cells using an intraductal approach. Innovation lies in the use of molding technology to produce branched channels of decreasing lumen size and identifying conditions that permit non-neoplastic mammary cell monolayer expansion and differentiation into a basoapically polarized epithelium on polydimethylsiloxane. This model will enable researchers to assess and direct the movement of particles to specific target cells. The production of this breast ductal system on-chip integrates expertise from biologists, engineers and biophysicists.

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areas of the ductal system. Indeed, the decreasing size of mammary channels provides too strong a resistance to the fluid movement necessary to collect the content of small distant channels. In addition ductal lavage is associated with discomfort and pain, and risk of perforation.[6] Mammary ductoscopy has been implemented as a breast cancer detection method and for assistance in surgery but improvement is still necessary to make it practical.[5,7] Fluid pressure would also prevent local therapies with intraductal antineoplastic drugs if original tumor sites were beyond reach.

One possible way to access all preneoplastic and tumor sites in the breast might be to use guided superparamagnetic submicron particles (SMP). The fluid can be left static if SMPs are moved through the breast ducts under magnetic fields of appropriate shapes and strengths. Iron-oxide SMPs would provide biocompatible and nontoxic paramagnetic material (*i.e.*, SMPs only acquire magnetic properties in the presence of a magnet);[8] these SMPs could also serve as both X-ray and MRI contrast agents. Moreover, fluorescently-labeled magnetic particles could serve to highlight ductal breast cancer cells for subsequent fluorescence-guided surgery. The SMPs can be engineered to target tumor cells and either, carry chemotherapeutic drugs, or trigger heat-induced cell death upon exposure to alternating magnetic field energy that can be readily transduced into the iron-oxide.

In order to set up meaningful experiments in ductal channels, to test targeting and toxicity of SMPs, it is critical to develop a cell culture system that mimics branched mammary channels. Ways to reproduce the mammary glandular system have been increasingly explored to provide an environment amenable to the better understanding of differentiation and tumor development. Murine cells have been successfully used in the past to recapitulate branching morphogenesis in the presence of epimorphine and growth factors.[9] However, this system does not permit the control of the number and size of ducts. Non-neoplastic human mammary epithelial cells have been used repeatedly to produce differentiated glandular units or acini, with cells basoapically polarized, thus mimicking the organization of the smallest mammary glandular structures but they usually fail to develop branched channels using classical 3D culture systems in the presence of basement membrane components.[10,11] Recent development in 3D culture design led to the formation of branched ducts from non-neoplastic breast epithelial MCF10A cells on a collagen I basis and/or silk protein scaffold and co-cultures with other cell types present in the breast.[12,13] However, like for branching obtained with murine cells, the size, number, and conformation of the branched ductal structures cannot be controlled and this system would be difficult to use for injection of SMPs. Moreover, MCF10A cells seldom make apically polarized epithelia due to the lack of tight junction formation at the apical side of cells.[11,14,15]

A mammary ductal system starts with a smaller diameter at the orifice, at the nipple, compared to the diameter measured a few millimeters inside the breast where it reaches 0.7 mm on average; then the diameter of the lumena decreases in size as branches form toward the terminal ductal lobular units (TDLUs).[3] In order to engineer a controlled ductal system with decreasing diameters, the optimal way might be to culture cells as a monolayer on preformed channels. Elastomeric Polydimethylsiloxane (PDMS) has been utilized to make patterning structures by soft lithography and can be coated with extracellular matrix (ECM) components to allow the culture of different cell types.[16] This substratum is most commonly used for microfluidic cell culture and its optical transparency and low autofluorescence properties permit high-resolution imaging through the material. Therefore PDMS appears like an optimal substratum to design branched ductal structures.

Here we have engineered a simple ductal system with branched channels of decreasing size using PDMS as the molded material. SMPs can be directed to move towards the smaller ducts and pulled out of the channels. With proper ECM coating, non-neoplastic HMT-3522 S1 mammary epithelial cells display basoapical polarity on PDMS and can be used to cover the channels.

Results

ECM-coated hydrophobic PDMS material is amenable to the phenotypically normal differentiation of breast epithelial cells

One of the challenges of mimicking the phenotypically normal organization of the breast epithelium is to reproduce the backbone of differentiation, the basoapical polarity axis.[11] Another challenge is to apply microscale cell culture necessary to mimic the size of the breast ducts to effectively produce mammary epithelial differentiation in a serum-free medium. In this case the goal is not to produce the well-known spherical three-dimensional (3D) structures referred to as acini, but rather a basoapically polarized monolayer of non-neoplastic cells organized into a tube. PDMS can be used to engineer differently shaped structures on which cells can be cultured.[16] However, it has not been applied yet to the culture of non-neoplastic mammary epithelial cells necessary to cover the walls of tiny channels.

The first step of our approach was to assess which ECM-based substratum permitted the expansion of the non-neoplastic human mammary epithelial HMT-3522 S1 cells cultured as a monolayer on PDMS. Engelbreth-Holm-Swarm (EHS) sarcoma-extracted ECM material has been commonly used to culture mammary epithelial cells; therefore we coated PDMS with EHS-based Matrigel™ and let it dry overnight before plating the cells. Using dried Matrigel™ should avoid the cell round-up normally obtained with the gel form. Over a 10-day culture period, PDMS surface modification using dried 5% Matrigel™ did not consistently foster cell monolayer expansion while dried 10 and 20% Matrigel™ led to the development of multicellular structures growing in 3D (Fig. 1A). However, PDMS surface modification with dried laminin 111, collagen IV, or a combination of laminin 111 and collagen IV, permitted expansion of the monolayer of S1 cells to 90% confluence within the same culture period. Interestingly, a drip of laminin 111 mixed with cells at the time of plating also led to monolayer expansion, providing a less cumbersome preparation of the cell culture environment (Fig. 1A). Growing monolayers of non-neoplastic mammary S1 cells

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on laminin 111-coated PDMS was not accompanied with any significant toxicity as shown by Trypan blue exclusion (Fig. 1B).

Immunostaining for the hemidesmosome component, α6-integrin, and the tight-junction protein, ZO-1, was used to assess proper localization of these basal and apical polarity markers, respectively. Cells cultured on dried or dripped laminin 111, and a combination of dried laminin 111 and dried collagen IV formed a basoapically polarized layer;

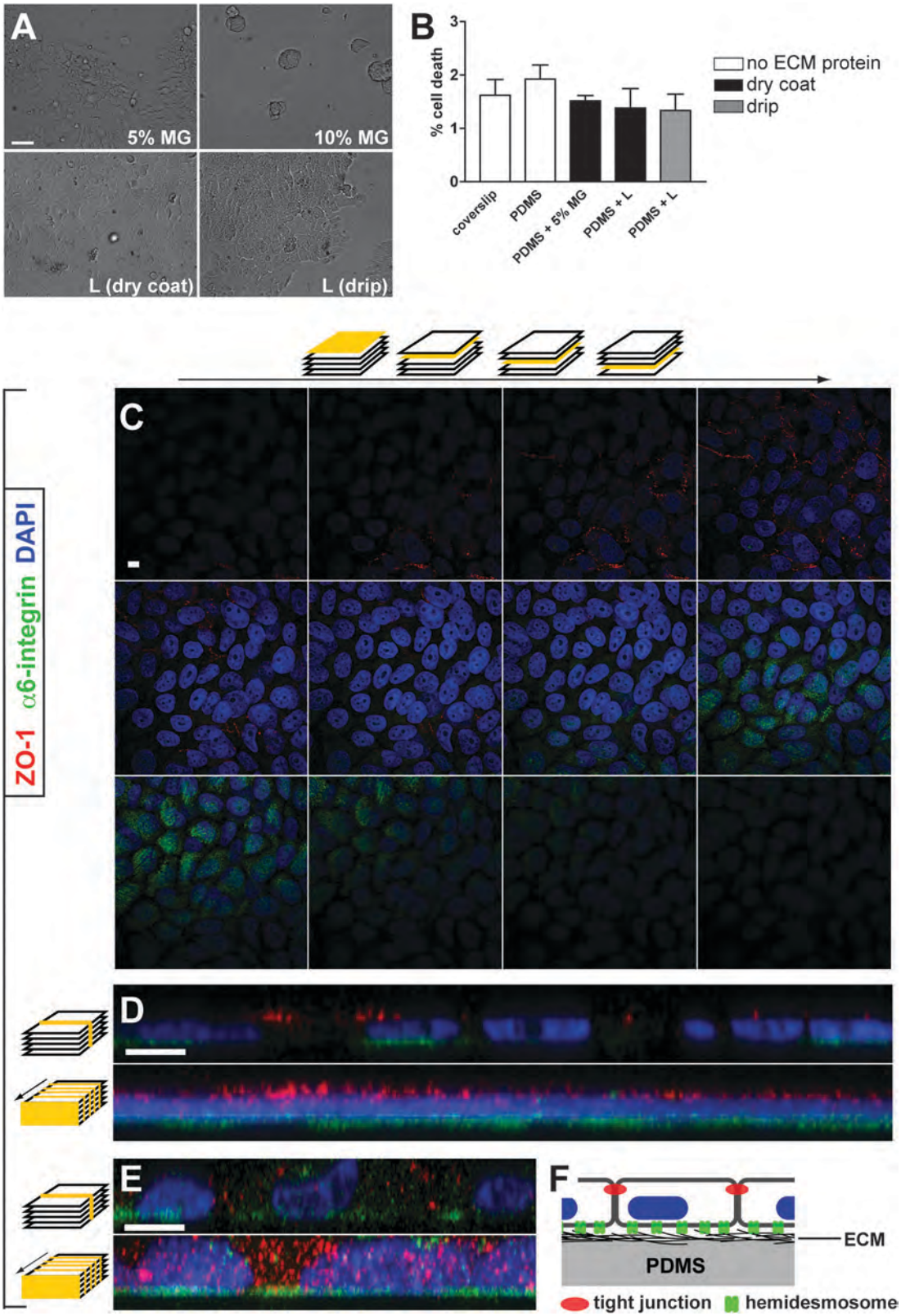

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Table 1 Basoapical polarity status depending on the substratum used for cell culture

	PDMS		Filter	
	Drip	Dry	Drip	Dry
5% Matrigel	n.d.	B/A*	n.d.	n.d.
10% Matrigel	n.d.	3D	n.d.	n.d.
20% Matrigel	n.d.	3D	n.d.	n.d.
Laminin 111	B/A	B/A	B/A	n.d.
Laminin 111 + collagen IV	n.d.	B/A	B/A	n.d.
Collagen IV	n.d.	n.d.	B/a	n.d.
Collagen I	n.d.	b/a	n.d.	n.d.

n.d., not determined; 3D, formation of three-dimensional multicellular structures; B, presence of basal polarity (based on α6-integrin marker); A, presence of apical polarity (based on ZO-1 marker); b, a, absence of basal polarity or apical polarity, respectively. (* nonconsistent results depending on replicate).

whereas no polarization was obtained with collagen I, used as negative control (Fig. 1C–E and Table 1). The gold standard for basoapical polarity of epithelial monolayers is to culture cells on filters.[17] The cells' display of basoapical polarity when cultured on PDMS with appropriate ECM was similar to cultures on filters dripped with laminin 111 or with a combination of laminin 111 and collagen IV (Table 1; ESI Fig. 1S§). Therefore PDMS surface modification with dried or dripped laminin 111 appears to be the simplest effective option to obtain a monolayer of basoapically polarized mammary epithelial cells.

Non-neoplastic mammary epithelial cells cover channels made of dried laminin 111-coated PDMS

A breast ductal system encompasses branches of decreasing lumen size, starting with the largest channels of 700 μm in average diameter[3] and ending at TDLUs with channels of 30 μm in average diameter. Soft lithography was used to design rectangle-shaped, PDMS branched channels of decreasing sizes from 50 × 120 μm, to 50 × 60 μm and 50 × 30 μm for height and width, respectively (see methods section) (Fig. 2A). Each channel of a particular width had a length of 5 mm and the whole ductal system could fit on a slide thus, enabling on-chip experiments. The tiny total surface of 7.1 mm^2 and total volume of 0.09 mm^3 of the on-chip ductal system may hamper the attachment and proliferation of non-neoplastic mammary epithelial cells. Therefore, two methods were compared for the culture of cells inside the channels. In the first method, cells were injected in closed channels using a metered injection system at 0.01 and 0.5 ml h^{-1}, and the medium was exchanged by diffusion by immersing the channel system inside a cell culture medium (Fig. 2B). In the second method, branched hemichannels (U-shaped) were engineered as described in the methods section and cells were cultured on this channeled surface in the presence of laminin 111 (Fig. 2B). Then the channels could be completed at room temperature by adding a PDMS membrane. To correct for the decrease in cell concentration inside the hemichannels or inside the complete channels and hence, the inhibition of cell division that would subsequently occur, the concentration of cells was increased 10 fold compared to usual, to reach 230 000 cells/cm^2.

In the first culture method, a laminin drip had to be used in the preformed channels since dried laminin was clogging some of the channels. Unfortunately, cells that were injected into the channels seemed unable to survive. In the second cell culture method, precoating the hemichannels with dried laminin 111 allowed cells to expand on the PDMS walls more effectively compared to laminin 111 dripped at the time of cell plating, without cells clogging the channel (Fig. 3A–D). Cells displayed basal and apical polarity, including those in the flat monolayer covering the side walls of the channels (Fig. 3E–F). Therefore, using such a small channel environment required the culture of cells on the hemichannels, before completing the ductal system with the PDMS membrane.

Fig. 1 Recapitulation of basoapical polarity on ECM-coated PDMS. HMT-3522 S1 cells were cultured on glass or on PDMS coated with dried Matrigel™ (MG at 19.2 and 38.4 μg total proteins/cm^2, corresponding to 5 and 10% of the stock solution, respectively), dried laminin 111 (L(dry coat), 5.2 μg proteins/cm^2), or dripped with laminin 111 (L(drip) at a final concentration of 133 μg ml^{-1}) for 10 days. (A) Bright field image of cultures of S1 cells. (B) Percentage of cell death measured with Trypan blue exclusion test. L = laminin, (Dunnett, $p > 0.05$, $n = 3$). (C)–(E) The distribution of apical polarity marker ZO-1 (red) and basal polarity marker α6-integrin (green) was analyzed by confocal microscopy in S1 cells cultured on PDMS coated with dried laminin-111 (C, D) or with collagen I as negative control for polarity (E). Nuclei were counterstained with DAPI (blue). Serial images from a *z* stack are shown in C; the direction of optical sectioning is indicated in the cartoon on top. (D) & (E), orthogonal ('side') views (top panels) and maximal intensity projections after reslicing in *xz* (bottom pannels); the orientation of the optical sections is indicated in the cartoon to the left. (F) Schematic representation of a polarized epithelial cell monolayer. Size bars, 50 μm (A) and 5 μm (C)–(E).

Fluorescent superparamagnetic submicron particles can be used with the PDMS-based channeled system and are nontoxic to the mammary epithelium

One of the goals of the ductal system on a chip is to provide a model to study the use of SMPs for future detection and treatment applications. SMPs of 0.86 μm in average diameter were loaded into complete cell-free microfluidic channels to assess their movement in this constrained environment. These relatively large particles were chosen so that individual particles can be visualized amidst live cells using phase and fluorescence microscopy. Also, SMPs will not require huge magnetic fields to move them since, all else being equal, the force driving the particles within the channels will be proportional to the cube of the diameter of the magnetic material portion of the particle. Furthermore, it is important that these SMPs be "superparamagnetic" (and not simply "paramagnetic") which means that they become magnetic only in the presence of a magnetic field. When the magnetic field is taken away the SMPs are free to disperse locally and, if targeted to surface markers on specific cells, bind to their targets. After injection, the SMPs showed Brownian movement, as expected. Upon application of the magnet, SMPs could be collected on one side of the channel and directed to enter specific channels of decreasing size (Fig. 4; ESI Movies 1–3§).

For toxicity assays, cells were cultured on PDMS coated with dried laminin 111 and incubated with different

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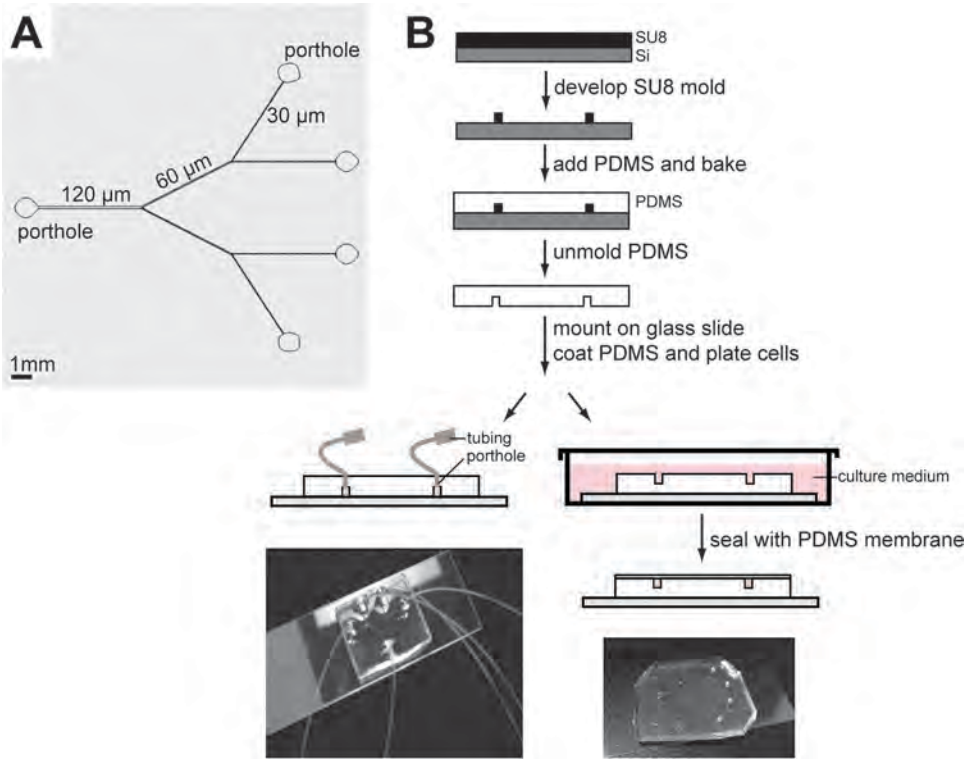

Fig. 2 Engineering of PDMS channels on a chip. A microchannel system was molded in PDMS, coated with laminin 111, and used as substrate for the culture of HMT-3522 S1 cells. (A) Schematic of the branched channel system. (B) Two independent approaches were developed: In the first approach (left, drawing and picture of the system), PDMS microchannels were sealed onto a glass coverslip, coated with dried or dripped laminin 111, and used for the culture of S1 cells in a closed environment. Cells were injected through tubing connected to the portholes using a syringe pump and the medium was changed by immersion. In the second approach (right), cells were cultured in an open 'hemichannel' system (top side of microchannel left open). The channels can be completed using a PDMS membrane on the day of the experiment.

concentrations of SMPs. A preliminary assay suggested that the solution containing SMPs was nontoxic, as shown by Trypan blue exclusion, when using a volume that corresponded to that added if SMPs were 10 fold the concentration of cells. This result was confirmed in several rounds of experiments showing no significant toxicity when incubating SMPs for two days in their solvent at 1, 5 and 10 fold the cell concentration at time of incubation. Confocal microscopy revealed that SMPs did not seem to go inside the cells, and there was no detectable effect of SMPs on basoapical polarity when used as a 10 fold concentration compared to cells, as shown by immunostaining for α6-integrin and ZO-1 (Fig. 5A–D). SMPs were used as raw materials in these experiments and therefore stuck nonspecifically to the cells and parts of PDMS not covered by cells. This close encounter between SMPs and cells was necessary to ascertain that the SMPs *per se* (*i.e.*, without addition of any specific targeting molecule) would not be affecting cells. Appropriate coating of SMPs with targeting molecules will be necessary to effectively control their travel to target cells upon application of a magnetic field in future experiments.

Discussion

We have shown that non-neoplastic mammary epithelial cells can cover laminin 111-coated hydrophobic PDMS and that the resulting epithelium displays basoapical polarity. This was a critical step in the design of the channel system as we have demonstrated previously that apical polarity is extremely labile and sensitive to culture conditions.[11] Indeed, the polarity status of the epithelium might impact future studies with antibody-coated SMPs necessary for targeting to specific cell types using cell surface markers. Laminin 111 was found to be sufficient to foster the development of basoapical polarity, confirming long-time knowledge that this ECM molecule acts as a powerful inducer of breast epithelial differentiation under different cell culture conditions.[18] Surprisingly, the EHS extract that contains more basement membrane ECM components than the sole laminin 111 could not provide consistent results. It is possible that the concentration of materials contained in this extract, some of which are not well determined, influences the spreading of cells and that it is more amenable for the formation of three-dimensional breast epithelial structures than flat monolayers of polarized cells.

A critical aspect of the design of a breast ductal system is to develop a model that contains branched channels of decreasing size. Based on our review of sections of normal looking breast tissue, the lumen size at the level of TDLUs should be around 30 µm in average diameter.[11] This is in the range of the channel size that was mimicked by the smallest branches of the ductal system built with PDMS if we take into account the thickness of mammary epithelial cells (~10 µm) cultured on the channels' surface. Producing such small channels was accompanied with technical challenges. Indeed, it is not surprising

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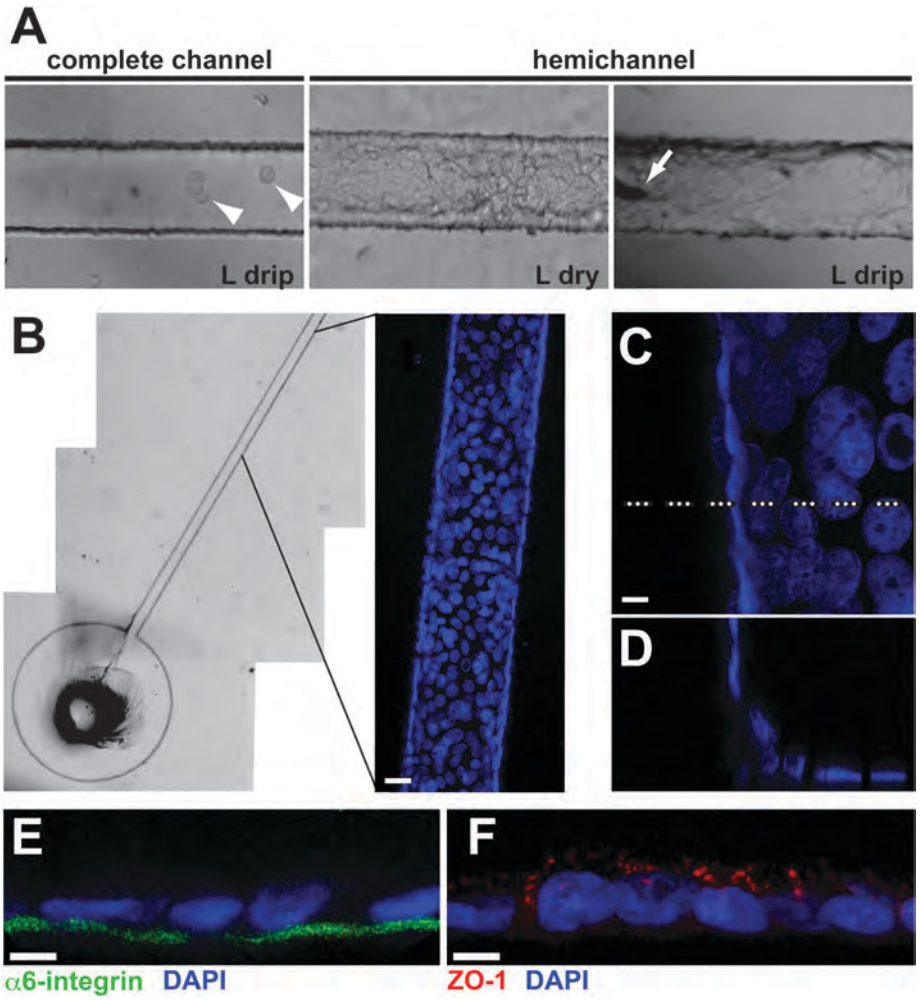

Fig. 3 Culture of mammary epithelial cells in branched PDMS hemichannels in the presence of laminin 111. HMT-3522 S1 cells were cultured in presence of laminin 111 (L) in complete microchannels or in hemichannels according to Fig. 2B. Laminin 111 was either coated and dried on PDMS (dry) or diluted in the H14 culture medium and dripped on the cell population at the time of plating (drip). (A) Bright field images. Individual round cells are indicated by arrowheads in a complete channel. Cell clumping in hemichannels with the drip method is indicated by the arrow. (B)–(D) Confocal analysis of DAPI-stained S1 cells in the terminal branch from a hemichannel coated with dried laminin 111. Maximal intensity projection of a *z*-stack taken at low magnification is shown together with tiled bright field micrographs of the hemichannel (B). Maximal intensity projection of a *z*-stack taken at high magnification (C) with orthogonal view at the level of the dotted line (D). (E) & (F) Confocal analysis of basal polarity marker α6 integrin (green) and apical polarity marker ZO-1 (red) in S1 monolayer located on a side wall of the hemichannel coated with dried laminin 111. Nuclei are counterstained in blue. Size bars, 20 μm (B) and 5 μm (C, E, F).

that our cells did not survive when injected in complete channels, likely as a consequence of shear stress. In other reports using cell injection in microfluidic systems, the entrance channel was much wider and the feeding system used parallel conduits.[19,20] Culturing cells in hemichannels (U-shaped) as successfully reported here, followed by the completion of the channel with a PDMS coverslip (the latter could be covered with cells), is an acceptable solution to deal with channels of very small diameter. Our results show that even in the smallest channels, the cells could effectively cover the hemichannel on all of its three surfaces without inducing intrachannel cell clogs (see Fig. 3). The absence of piling up of cells responsible for clogs in the hemichannels was observed with dried laminin 111; however, clogs were present when laminin 111 was dripped, although on PDMS coverslips both dried and dripped laminin 111 led to the development of a flat monolayer of cells. This might be due to the narrow indent used to create the hemichannel, which could entice cells to pile up more easily when surrounded by dripped laminin 111. Importantly, with hemichannels, it will be possible to seed tumor nodules within the ductal system at specific locations to study SMPs targeting.

In this model of a breast ductal system we did not include myoepithelial cells at the basal side of the luminal cells nor terminal ductal lobular units at the ends of the narrowest channels although these represent normal features of the mammary epithelium. Instead, we focused on the production of a basoapically polarized monolayer of luminal cells because this device is intended to mimic the luminal portion of the ductal breast system. One of our goals is to introduce tumor cells and nodules in the microchannels that mimic the ductal breast system, the development of which is reported here, to

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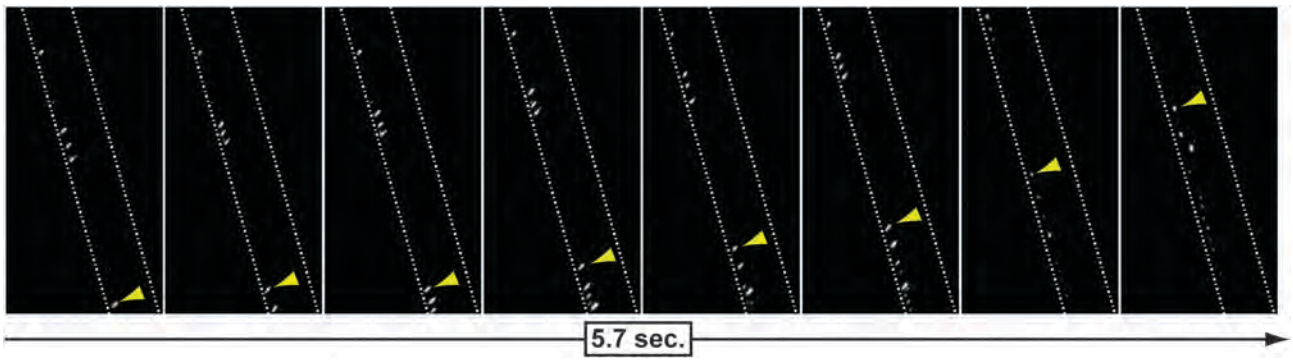

Fig. 4 Movement of fluorescent magnetic submicron particles within channels. Time lapse micrographs showing the movement of SMPs in a 50 × 30 μm (height × width) channel. A pulling force was exerted using a wedge shaped magnet. The arrowhead points to one particle followed through the images taken at 700 ms intervals.

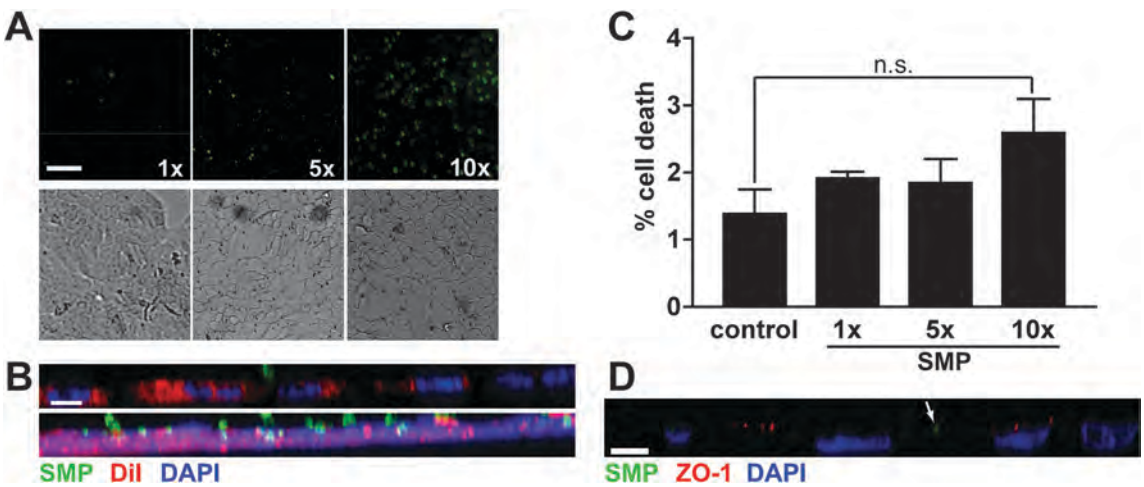

Fig. 5 Lack of toxicity of fluorescent magnetic submicron particles. SMPs were incubated for 48 h with S1 cells cultured on PDMS coated with dried laminin 111 at a concentration of 1, 5, or 10 particles per cell. (A) Fluorescent SMPs (green, top panel) and corresponding bright field images of cells (bottom panel). (B) Orthogonal view from a confocal *z*-stack showing cell nuclei (DAPI, blue), cell membranes (DiI, red) and fluorescent SMPs (green). The maximal intensity projection after reslicing in *xz* is shown at the bottom. (C) Percentage of cell death measured by Trypan blue staining after incubation of S1 cells with the SMPs. (Dunnett, $p > 0.05$, $n = 3$). (D) Orthogonal view of immunostaining for apical polarity marker ZO-1 (red) in S1 cell monolayer incubated with SMPs (10 particles per cell, green, arrow). Size bars, 50 μm (A), 5 μm (B and D). n.s., nonspecific.

test SMPs for neoplasia detection and treatment purposes. SMPs were found to travel effectively under the power of a custom wedge shaped magnet. Critically, no cytotoxity or effect of the SMPs on the differentiation of the breast epithelium, as measured by the distribution of basoapical polarity markers, could be detected even after two days of incubation.

The proposed model of the mammary ductal tree will be an asset to the initial steps of the design of nanomedicine techniques. For instance, magnetic fields could be used to provide a force to move antibody-targeted fluorescent and large SMPs through static fluid channels covered by mammary epithelial cells. In the long-run the SMPs will be designed to be "theranostic" meaning that they can be used for both diagnostic and therapeutic purposes. The SMPs could serve as X-ray contrast agents that will enhance mammograms, providing greater contrast between tumors and fibrous breast tissue. In principle, if SMPs are targeted against specific surface markers of neoplastic cells in the ducts, free SMPs can be "washed" away, by reversing the direction of the magnetic field, to leave only SMPs specifically bound. The SMPs bound to neoplastic cells can also serve as Magnetic Resonance (MR) imaging contrast agents, possibly enabling higher resolution MRI to determine the exact 3D location, size, and volume of the tumor for subsequent surgery. Since the SMPs are also fluorescent, they could be used to guide surgeons for real-time fluorescence-guided surgery to more completely remove tumors that may not be visible under normal surgery. Additionally, the SMPs could also contain anticancer agents that could be delivered directly to the tumor, hence decreasing the patient's exposure to total-body chemotherapy. All of these are examples of applications of SMPs for the fight against breast cancer, and the breast ductal system that we have developed will be a critical stepping stone to the development of effective SMPs.

Methods

Cell culture

Non-neoplastic human mammary epithelial cells (HMT-3522 S1[21]) were cultured at 37 °C in 5% CO_2 in H14 medium consisting

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of DMEM/F12 (Sigma, St Louis, MO) with 250 ng ml^{-1} insulin (Boehringer Mannheim, Indianapolis, IN), 10 μg ml^{-1} transferrin (Sigma), 2.6 ng ml^{-1} sodium selenite (BD Biosciences, Bedford, MA), 10^{-10} M estradiol (Sigma), 1.4 μM hydrocortisone (BD Biosciences), 5 μg ml^{-1} luteotropic hormone (Sigma), and 10 ng ml^{-1} epidermal growth factor (EGF; BD Biosciences) for 10 days with medium changed every two to three days.

Laminin 111 and collagen IV (BD Biosciences, Discovery Labware) were used at a final concentration of 133 μg ml^{-1} and 20 μg ml^{-1}, respectively, shown to induce polarity in S1 cells.[11] When let to dry overnight, the laminin 111 coat corresponded to 5.2 μg proteins/cm^2 of PDMS. Matrigel™ (BD Biosciences Discovery Labware) was used at a final concentration of 5, 10 or 20% (corresponding to 19.2, 38.4, or 76.8 μg total proteins/cm^2, respectively). When dried, these ECM substrata were placed at 37 °C overnight before plating cells. When laminin 111 and collagen IV were combined, they were dried sequentially (starting with laminin 111) over two consecutive nights. For the drip method, the substrata were mixed at the desired final concentration in 50% of the final volume of the medium and dripped over the cells plated a few minutes before in 50% of the final volume of the medium.

Toxicity test

S1 cells were cultured directly on PDMS coverslips covered with different ECM substrata for 10 days to assess toxicity. For experiments involving SMPs, S1 cells were cultured for eight days with dried or dripped laminin 111 before incubating for 48 h with 1, 5 and 10 times as many SMPs (Catalog # ME03F, Bangs Laboratories, Fishers, IN) as S1 cells present on day 8, or with the SMP-free preserving solution used at a volume corresponding to 10, 5 or 1 times as many SMPs. The number of cells at day 8 was estimated by detaching and counting cells from one well of the 12-well plate used for the experiment. For the toxicity tests, after medium removal, cells were incubated with Trypan blue (Sigma) for five minutes and washed in PBS. Blue-stained dead cells were scored out of a total of 500 cells.

Fluorescence immunostaining

S1 cells were incubated for 10 min in a permeabilization buffer [0.5% Triton X-100 in cytoskeleton buffer (100 mM NaCl, 300 mM sucrose, 10 mM pipes, pH 6.8, 5 mM $MgCl_2$)] with protease and phosphatase inhibitors [1 mM Pefabloc (Roche Diagnostics, Indianapolis, IN), 10 μg ml^{-1} aprotinin (Sigma), 250 μM NaF], before fixation in 4% paraformaldehyde (Sigma) and immunostaining as previously described.[22] Primary antibodies were polyclonal rabbit anti-human ZO-1 (Invitrogen, Carlsbad, CA) and monoclonal rat anti-human α6-integrin CD49f (Millipore, Billerica, MA). Secondary antibodies were donkey anti-Rabbit IgG (H + L) and donkey anti-Rat IgG (H + L) (Jackson ImmunoResearch, West Grove, PA). Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI; Sigma) and specimens were mounted in ProLong® Gold antifade reagent (Invitrogen).

PDMS handling and making of channels

Photoresist masks were made on 4″ silicon wafers following standard photolithographic procedures. Briefly, SU-8 2025 (Microchem, Newton, MA) was spun to a 50 μm depth onto a cleaned 4″ silicon wafer. The photoresist was exposed to the designed mask and then developed. Further procedural details were as described previously.[23] The completed wafer was baked and treated with 97% trichlorosilane vapors for 30 min. PDMS elastomer and curing agent (Dow Corning Sylgard 184, Ellsworth Adhesives, Germantown, WI) were mixed in a 10 : 1 ratio and debubbled for 20 min. The PDMS was then poured over the silicon–photoresist mold and cured overnight at room temperature. Individual chips were cut from the mask and a conical 20 gauge needle was used to bore holes for tubing. A 10 : 1 ratio of PDMS to curing agent was also spread thinly on a 5″ Petri dish and cured overnight at room temperature. The thin PDMS was cut to cover a large rectangular coverslip as the base of the chip. Both the PDMS coated coverslips and the PDMS chips were oxidized using a Corona treater. The PDMS surfaces were exposed to the localized oxygen plasma for approximately 30 s each. To make complete channels, each chip was pressed together with a coverslip and any bubbles were rubbed out. The bound chips were then baked at 70 °C for 30 min to stabilize the bond. Tygon tubing was inserted into the inlet and outlet ports and sealed with 10 : 1 ratio uncured PDMS.

SMP handling and coating

Fluorescent superparamagnetic microspheres (0.86 μm average diameter) were chosen to be large enough to be moveable with a magnetic field (the amount of ponderomotive force provided by the external magnetic field is directly proportional to the volume of the magnetic material in the SMP), reduce random Brownian movement, and permit observation with conventional fluorescence microscopy (*i.e.*, diameter above the optical imaging resolution limit). The SMPs were injected into the PDMS channels using a 1 ml syringe and 30 gauge blunt tip needle. Once the SMPs were in the channels, flow was allowed to stop as no further pressure was applied to the syringe. A permanent magnet (Quadrant Magnetics, Louisville, KY) with NdFeB ("Neo 42") material with a target poleface field of approximately 300 mT was used to drag the SMPs through the channels.

Imaging

Bright field images of cell monolayers were taken with an Olympus IX70 epifluorescence microscope (Olympus, Center Valley, PA). Fluorescently stained samples were analyzed using a Zeiss (Oberkochen, Germany) LSM 710 confocal microscope system equipped with a 100×/1.4NA oil immersion objective. Images were captured using the Zen software (Zeiss) and processed with ImageJ (http://rsbweb.nih.gov/ij/index.html). Video recording of SMP movements was performed on an inverted fluorescence microscope (Nikon Diaphot) at 20× magnification using a Retiga EXi camera (QImaging, Surrey, BC, Canada). The Image-Pro Plus software was used to capture images every 50 ms.

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Statistical analyses

Data are presented as means ± SEM and statistical comparisons were performed using GraphPad Prism 3.0 software (GraphPad Software Inc, San Diego, CA). Nonpaired *t*-test was used for comparison of two groups whereas one-way ANOVA with Dunnett post-hoc test was employed for comparisons between three or more groups of samples. A $p < 0.05$ was considered significant.

Conclusion

This first attempt to reproduce the mammary ductal system *in vitro* demonstrates the importance of combining engineering and biology expertise in order to achieve appropriate settings for epithelial differentiation in a defined environment, *i.e.*, here the tiny branched channels. Our ultimate goal is to use this breast-on-chip system to coculture phenotypically normal and diseased cells and tumor nodules, and assess the targeting of SMPs to specific cells while these particles migrate within ducts that mimic the luminal breast environment.

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